Pergamon

Environment International, Vol. 25, No. 6/7, pp. 805–817, 1999

0160-4120/99/$–see front matter

PII S0160-4120(99)00055-0

A BIOMATHEMATICAL MODEL OF HEMATOTOXICITY

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EI 9903-144 M (Received 30 March 1999; accepted 5 June 1999)

Pharmacodynamic models representing interactions among chemicals and cells can help to clarify how time patterns of administered dose affect risks of adverse health outcomes. This paper summarizes a model that predicts the effects of the myelotoxic and immunosuppressive drug cyclophosphamide (CP) on the hematopoietic (blood-forming) system. It consists of a set of physiological compartments representing hematopoietic progenitor cell, granulocyte-macrophage (GM)-committed stem cells, and more mature blood cells. These compartments are linked by nonlinear feedback control loops and are susceptible to first-order cell-killing kinetics from cytotoxic metabolites. The model was validated by testing its predictions against experimental and clinical data for blood cell counts following administration of CP to mice, dogs, and humans. It successfully explains apparent anomalies and patterns in previously published data, including the fact that smaller cumulative doses can cause larger hematotoxic responses. An intriguing prediction from the model is that sustained exposures to sufficiently small concentrations of myelotoxic agents may tend to provoke a protective response, increasing rather than decreasing the numbers of GM colony-forming units (CFU-GM) and early hematopoietic stem cells available to sustain hematopoiesis. ©1999 Elsevier Science Ltd

INTRODUCTION

Suppose that a risk manager must choose among the following risk mitigation options for protecting workers from a potentially hazardous airborne chemical in a manufacturing plant:

1) reduce daily occupational exposure time from 8 h to 4 h;
2) reduce 8-h average workplace concentration by 50%;
3) reduce weekly exposure from 5 d to 2.5 d; and
4) reduce yearly exposure from 50 to 25 weeks per y.

Which option is most health-protective? By how much would each be expected to reduce the health risk due to occupational exposures? How well can these questions be answered without further specifying the options, e.g., how the 4 h in option 1 are distributed within the work day, how the 25 weeks in option 4 are distributed within the year, and so forth? This paper shows how biomathematical, biologically-motivated risk assessment models can address such questions.

Dynamic dose-response models

The need for explicit dynamic models in quantifying dose-response relations is often implicitly denied in applied risk assessments. Instead, dose metrics, such as cumulative exposure or cumulative dose per unit body weight or per unit surface area, are commonly used to equate the risks of different exposure histories. Dose metrics are used to extrapolate risks from high to low doses and from one species and strain to others. Uncertainties about the validity of the extrapolation are often equated to uncertainty about the most appropriate choice of dose metric.

Table 1. Results of a stop-exposure experiment for isoprene in male B6C3F1 mice.

Group	ppm**	weeks	h/d	Liver adenomas	Lung adenomas	Other adenomas	Liver carcinomas	Lung carcinomas	Histio-sarcomas
1	0	0	8	0.22	0.22	0.14	0.18	0	0
2	10	80	8	0.24	0.32	0.12	0.12	0.02	0.04
3	70	40	8	0.29	0.16	0.30*	0.22	0	0.04
4	70	80	8	0.30	0.08	0.18	0.18	0.04	0.04
5	140	40	8	0.44*	0.20	0.28*	0.20	0.02	0.02
6	280	20	8	0.36	0.32	0.36*	0.24	0.06	0.16*
7	2200	80	4	0.42*	0.30	0.56*	0.30	0.06	0.14*
8	2200	40	8	0.57*	0.59*	0.65*	0.37*	0.06	0.14*

Explanation: Columns 2-4 summarize the exposure factors defining each dose group. The remaining columns show the fraction of animals in each dose group that were found to have each tumor type at necropsy.
* Tumor incidence rates marked with an asterisk are significantly greater than in the control group ($p < 0.05$ by Fisher's Exact Test).
** ppm = μL/L.

In conjunction with another widely used assumption – that excess risk at sufficiently low doses increases in approximate proportion to the dose metric – the dose metric approach provides a simple, clear method for assessing the risk consequences of risk management options such as 1-4. For example, these principles imply that all four options reduce health risk equally at small concentrations. Different practitioners might debate which specific parametric risk model is most appropriate, e.g., logistic regression, probit, proportional hazards, linear relative risk, linear absolute risk, and so forth. Yet, the rank-ordering of risk management options and estimates of their relative quantitative efficiencies in reducing risk do not depend on such model differences. They are fully determined by the assumptions of a dose metric and approximate low-dose linearity. This may create an apparent consistency and robustness in the results of different specific parametric risk models, including all of those just listed, that are traceable to their common underlying methodological approach.

Despite its simplicity and robustness, the dose metric modeling framework is inconsistent with experimental data that permit it to be tested, for some chemicals of practical interest. Stop-exposure experiments, which expose different groups of animals to chemical carcinogens for different amounts of time, reveal results that seem to contradict the most basic tenets of applied risk assessments based on dose metrics. For example:

1) Smaller cumulative doses may produce larger toxic and carcinogenic responses, exemplified by Luke et al. (1988a), for benzene cytotoxic effects on erythrocytes in mouse bone marrow and Cox et al. (1996) for isoprene effects on tumors at multiple anatomic sites in mice.

2) Relatively small increases in concentration can dramatically increase tumor incidence, exemplified by Williams et al. (1993), for hepatocarcinogenesis and Melnick et al. (1990) for butadiene-induced lymphomas.

3) Extending durations of exposure may have little or no impact on tumor risks as described by Cox et al. (1996) for isoprene-induced tumors at several anatomic sites.

4) Sufficiently low exposure concentrations can have disproportionately small impacts, or even appear to have beneficial effects, prompting some investigators to speculate about biological hormesis (David and Srendsgaard 1990).

Table 1 illustrates some of these phenomena for isoprene. The following patterns are noteworthy:

1) Doubling concentration from 70 ppm (μL/L) to 140 ppm (μL/L) between exposure groups 3 and 5 increases liver adenomas from 0.29 to 0.44. But, doubling time of exposure from 40 weeks to 80 weeks between exposure groups 3 and 4 does not increase risk significantly at any site, and even appears to reduce it. This observation is not explained by competing risks of death from toxicity, as animals tolerated these concentrations. Nor is it explained by saturation of carcinogenic response mechanisms, as higher concentrations produce much higher tumor yields. Thus, no dose-response theory that predicts that risk increases with cumulative exposure provides a useful model for these data.

Table 2. Results on benzene hematotoxicity and genotoxicity.

End-point	Result	Reference
Hematotoxicity in mouse bone marrow, measured by a variety of indicators	10 ppm* x 8 weeks has no hematotoxic effect but 100 ppm* x 1 week has a marked hematotoxic effect	Farris et al. 1997
Hematotoxicity in mouse bone marrow, indicated by changes in CFU-GM and spleen CFU-S stem cell populations	10 ppm* x 10 weeks has no myelotoxic effect in mice, but 100 ppm* x 1 week greatly suppresses CFU-GM and CFU-S	Green et al. 1981a; 1981b
Hematotoxicity in mouse bone marrow, measured by reduction in CFU-GM per tibia bone marrow	21 ppm* of benzene x 6 d (= 3,024 ppm*-h) had a smaller hematotoxic effect than 50 ppm* x 2 d (2400 ppm-h)	Toft et al. 1982
Hematotoxicity in mouse bone marrow, measured by reduction in CFU-GM per tibia bone marrow	95 ppm* of benzene x 2 h/d, 5 d/week x 2 weeks has little hematotoxic effect. Yet, 96 h of continuous exposure to 21 ppm* produces severe hematotoxicity. (Both are about 2000 ppm*-h)	Toft et al. 1982
Hematotoxicity in mouse bone marrow, measured by erythropoiesis	Benzene metabolites (HQ and phenol) synergize strongly in suppressing murine erythropoiesis	Guy et al. 1990; Chen and Eastmond 1995; Chen et al. 1994
Genotoxicity in mouse bone marrow erythrocytes, measured by induction of micronuclei in polychromatic erythrocytes (MN-PCE)	HQ and phenol synergize in creating micronuclei (MN) in erythrocytes	Chen and Eastmond 1995; Barale et al. 1990; Marrazzini et al. 1994
Genotoxicity in human lymphocytes in vitro, measured by clastogenicity	HQ and catechol synergize strongly (7 x additive effect) in creating genotoxic damage in cultured human lymphocytes	Robertson et al. 1991
Genotoxicity in mouse bone marrow erythrocytes, measured by MN-PCE	MN-PCE response is insensitive to duration of exposure and follows a quadratic dose-response curve below 200 ppm*	Toft et al. 1982; Tice et al. 1989; Luke et al. 1988b Farris et al. 1996
Genotoxicity in mouse bone marrow in vivo, measured by chromosomal aberrations	Doubling administered dose from 440 to 880 mg/kg increases chromosomal aberrations 8-fold	Ciranni et al. 1991

*1ppm =μL/L.

2) Quadrupling exposure concentration from 70 ppm (μL/L) to 280 ppm (μL/L) while quartering exposure duration from 80 weeks to 20 weeks unambiguously increases tumor risk (compare exposure groups 4 and 6), even though cumulative exposures are identical. Thus, no risk model that uses cumulative exposure (area under curve (AUC)) as a dose metric adequately describes these data.

3) At higher concentrations, doubling h/d of exposure while halving weeks of exposure increases the risk of adenomas and liver carcinomas (compare exposure groups 7 and 8), even though cumulative exposures are identical.

4) Liver and lung tumors (both adenomas and carcinomas) are only significantly elevated at concentrations above 70 ppm (μL/L). In this data set, 140 ppm (μL/L) is the smallest concentration for which significant increases were observed.

Such observations challenge any dose-response model that uses a dose metric in which risk increases monotonically and/or symmetrically with exposure concentration and duration.

Stop-exposure experiments for health end-points other than cancer show similar patterns. Genotoxic, cytogenetic, and cytotoxic responses are often sensitive to the time pattern of dose administration, rather than only to the AUC of administered dose (or of metabolites formed). For example, Table 2 summarizes experimental results for benzene, a relatively well-studied human leukemogen and animal carcinogen, for endpoints other than (and perhaps causally prior to) cancer. Benzene metabolites such as phenol, hydroquinone (HQ), and catechol synergize strongly in producing both cytogenetic and cytotoxic damage. Since carcinogenesis is often postulated to arise from the combination of genotoxic and cytotoxic effects (Farris et al.

1997 for benzene; Williams et al. 1993 for the hepatocarcinogen DEN; Monticello and Morgan 1994 for formaldehyde), it is not surprising that chemicals with strongly nonlinear dose-response patterns for genotoxic and cytotoxic effects may exhibit similar nonlinear patterns for tumors.

These examples demonstrate the need for dose-response models that do not satisfy the usual assumption that risk is an increasing function of a dose metric that combines exposure concentration and duration in a simple, monotonic, and perhaps symmetric way. The following sections investigate the potential of biologically-motivated models, which attempt to simulate key dynamic aspects of adverse health effects, to obtain more realistic and useful predictions.

METHODS

To assess how well biologically-motivated models can predict and explain phenomena such as those in Tables 1 and 2, the following three steps were carried out:

1) A dynamic simulation model of pharmacokinetics and cytotoxicity/cell kinetics was constructed for a well-studied leukemogen (cyclophosphamide (CP)). Mathematical models of pharmacokinetics and metabolism, hematopoiesis (blood formation), and hematotoxicity and myelotoxicity (blood-poisoning and marrow-poisoning) were based on previously published data and modeling efforts for CP, as described below. They were combined and implemented in a continuous simulation model using the ITHINK modeling environment (ITHINK 1997).

2) The model's predictions were validated with data from several species and agents. Model parameters estimated primarily from canine data were used successfully to predict observed dynamic responses in humans, dogs, and mice exposed to CP. Interspecies extrapolations were made by adjusting only two scaling parameters – a time-scaling factor and a dose-scaling factor – to account for differences in body weight and metabolism. Similar work recently validated similar model predictions for effects of radiation on human and canine hematopoiesis (Fliedner et al. 1996; Tibken and Hofer 1995).

3) The validated model was applied to explain and predict the dynamic responses of specific cell populations to different time patterns of dosing with hematotoxic agents. Dynamic responses were simulated for specific hematopoietic progenitor cell populations (e.g., early colony-forming units granulocyte-macrophage (CFU-GM)) thought to be involved in chemically-induced myeloid leukemogenesis (Irons and Stillman 1996).

Structure of the biologically-motivated model

The biomathematical model of hematotoxicity is described in Cox (1996), which gives detailed model equations in an appendix. It is based on the model of Steinbach et al. (1980) with some simplifications and improved CP pharmacokinetics based on experimental data (Sladek 1988).

The model consists of the following components:

1) A simple linear compartmental flow model (Jacquez 1997) describes the pharmacokinetics and metabolism of CP in humans. The model structure and micro-constants describing CP pharmacokinetics and metabolism are based on measurements in human patients, which are well approximated by a classical two-compartment model (Cohen et al. 1971; Sladek 1988). Because pharmacokinetics and metabolism are approximately linear, the AUC per unit time for any CP metabolite in any compartment is approximately proportional to the AUC-per-unit time of administered CP. This proportionality justifies using a simple classical compartmental model, since it is likely that a more realistic and sophisticated description of metabolism and pharmacokinetics (e.g., a PBPK model) would not noticeably change predictions for hematotoxic responses.

2) A nonlinear compartmental flow model with feedback-control loops describes the myelotoxicity and hematotoxicity of CP. This model consists of the following six main sequential compartments, describing the GM lineage of the hematopoietic system: 1. early stem cells and hematopoietic progenitor cells (HPCs); 2. granulopoietic committed stem cells (e.g., CFU-GM); 3. proliferative cells (myeloblasts, promyelocytes, myelocytes); 4. maturation pool; 5. bone marrow reserve; and 6. peripheral blood granulocytes.

Each compartment is fed by its predecessors and may provide feedback to them via control signals (e.g., representing the cytokine network). Partial differential equations (PDEs) describe the dynamics of the age-structured CFU-GM cell populations and later proliferative populations. The simulation model approximates these PDEs numerically by systems of ordinary differential equations (ODEs) (10 serial subcompartments) to make the mean and variance of cell transit times conform to experimentally observed values in dogs (Steinbach et al. 1980).

Feedback control. Nonlinear feedback loops, implicitly modeling the regulatory effects of the cytokine network, feed back information (interpreted as strengths of regulatory signals) from blood cell population sizes to control the following rate parameters: 1. fraction of early HPCs that differentiate instead of self-renewing; 2. rate of recruitment of resting stem cells into active cycling; 3) birth rates of CFU-GM and downstream proliferative cells; and 4) release rate of mature granulocytes from the bone marrow reserve to the peripheral blood.

These feedback control laws are described by smooth curves that were constructed to pass through experimental data points. Typically, the experimental data consisted of extreme values (e.g., maximal and minimal cell division rates observed under a variety of experimental conditions) with some intermediate data points. A parametric s-shaped function was then used to interpolate among these observed values, with its parameters being estimated by least squares. Most of the parameter values for the CP cytotoxicity and cell kinetics component were taken from previously published estimates based on a model of granulopoiesis in dogs (Steinbach et al. 1980). The appendix of Cox (1996) details the functions and parameter values used.

Simulation framework. The combined CP pharmacokinetics and hematotoxicity model is a continuous simulation model of the standard form (van der Bosch and van der Klauw 1994):

$$\mathbf{dx}(t)/dt = \mathbf{f}[\mathbf{x}(t), \mathbf{b}(t)]$$

$$\mathbf{b}(t) = \mathbf{g}[\mathbf{x}(t)]$$

where,

$\mathbf{x}(t)$ is a state vector with components describing 1) the quantity of CP and its metabolites in each compartment of the pharmacokinetic model and 2) the number of cells in each compartment of the cell kinetics and cytotoxicity model at time t; and

$\mathbf{b}(t)$ is a vector of rate parameters. Its components are constants for the pharmacokinetic model and are functions of the cell population sizes for the cell kinetics and cytotoxicity model.

The vector function $\mathbf{b}(t)$ also includes cytotoxic parameters describing the rate of cell-killing of cycling HPCs and CFU-GM cells per unit concentration of toxic CP metabolites (specifically, phosphoramide mustard) in these marrow cell populations. The feedback laws determining $\mathbf{b}(t)$ from $\mathbf{x}(t)$ are symbolized by the vector function g and are detailed in Cox (1996). Initial values for cell population sizes were taken from published experimental data, as in Steinbach et al. (1980), and starting values for CP in different compartments were assumed to be zero. Given the initial conditions $\mathbf{x}(0)$ and any specified dosing history, the simulation model determines the resulting histories of CP and metabolite concentrations and fluctuations in hematopoietic cell populations by numerical integration of the simulation model equations.

Model validation methods

The biologically-motivated hematotoxicity model was validated for CP by comparing its predictions to data not used in creating the model. The empirical validation consisted of the following two efforts:

1) *Validation in humans:* Using the model formulated above for dogs and a simple adjustment for interspecies dose conversion, the effects of repeated CP treatments on humans were predicted. The model predictions were compared to published clinical data to assess its predictive validity for humans.

2) *Validation in mice*: Using the above model and the same simple adjustment for interspecies dose conversion, the effects on hematopoiesis (CFU-GM and peripheral granulocytes) of single and multiple injections of CP in mice were predicted. The predictions were tested by carrying out these experiments.

Fliedner et al. (1996) and Tibken and Hofer (1995) also validated a similar model for radiation effects on hematopoiesis in dogs and humans.

Interspecies extrapolation. The model of Steinbach et al. (1981) was developed for dogs, for which it appeared to have useful descriptive validity. To test the validity of the modified model in the present paper, its predictions were compared against experimental observations in mice and humans.

Since humans have a larger body weight than dogs, their physiological processes are expected to run more slowly. The most parsimonious way to adjust for this effect is to dilate the time axis, multiplying it by a constant greater than 1 to slow down predicted canine hematotoxic responses (e.g., changes in cell population sizes) to predict the timing of corresponding human responses. Rather than using allometric arguments to estimate the scaling factor, it was estimated directly by comparing the results of analogous experiments in dogs and humans. Comparing the time course of peripheral white blood cells (WBCs) in canine model simulations

following a single injection of CP (Steinbach et al. 1980) to the time course reported for humans (Coggins et al. 1960) showed that the nadir occurs at approximately 11 d in humans compared to approximately 7.5 d in dogs. Thus, predictions from the canine model are scaled by a factor of 11/7.5 = 1.47 along the time axis to obtain predictions for humans. The pharmacokinetics submodel was already developed from human data (Cohen et al. 1971), and hence requires no rescaling. Also, rescaling its relatively fast dynamics would make little or no difference to the slower CP-hematotoxicity submodel for most dosing scenarios.

For mice, a contraction of the time axis must be made. Comparing experimental data on murine hematotoxic responses to CP injections (DeWys et al. 1970) to human clinical data (Bergsagel et al. 1968; Buckner et al. 1972; Nissen-Meyer and Host 1960) suggested that the time scale for hematopoietic responses in mice should be contracted by a factor of about 0.4 compared to humans. This estimated adjustment factor was used in making all predictions for mice.

Mice have a higher ratio of surface area-to-volume than do dogs or humans. Therefore, the administered mg/kg required to produce a given dynamic response in mice (e.g., a given depth of the nadir as a percentage of the normal level) may be different from the mg/kg required to produce the same response in dogs or humans. The required dose-scaling factor was estimated as approximately 5, based on the observation that mouse responses to 300 mg/kg (DeWys et al. 1970) appear to correspond roughly to human responses to 60 mg/kg (Coggins et al. 1960), appropriately speeded up. However, responses within each species to all sufficiently high single doses are similar enough to each other that the exact correspondence between mouse and human dose scales is hard to identify from previously published data. While it might in principle be desirable to also account for differences between dogs and humans in the hematotoxic potency of CP, the high levels of CP used in clinical trials approximately saturate the hematotoxic response, making detailed potency adjustments unnecessary.

Model validation tests. The complete CP model contains over 120 individual equations and formulas and over 20 parameter values, all with values estimated from experimental data. To validate its assumptions and implications, the following steps were taken:

1) *Face validity*. Face validity and internal consistency of the model were tested by introducing deliberate errors into its equations (e.g., using incorrect formulas or parameter values) and confirming that the resulting outputs became unrealistic, e.g., unstable in the absence of any external perturbation from dosing. These efforts revealed that the self-consistent dynamic behaviors exhibited by the model (specifically, homeostasis and a stable steady state) do not survive introduction of such obvious errors, increasing confidence that they have not been made.

2) *Descriptive validity*. Descriptive validity of the original Steinbach et al. (1980) model was established by checking that its predictions for HPC stem cells in bone marrow and for WBC counts after an injection of 15 mg/kg CP agree with experimental observations on dogs (Steinbach et al. 1980). These same experimental data points had been used to estimate the model parameters, however, so this check only confirms the descriptive validity of the model, i.e., its ability to explain a large number of data points (over 70) with a much smaller number of parameters. It does not prove that other parameter values would not fit the same experimental data equally well but make different predictions outside the range of observed experimental conditions.

3. *Predictive validity*. Predictive validity of the CP model was first assessed using human data by simulating the outcomes of clinical experiments previously reported in the literature (Buckner et al. 1972; Nissen-Meyer and Host 1960) and comparing the model-predicted time courses of blood cell counts to corresponding clinically observed values. The human data sets and clinical trials used to test the model are quite different from the animal experiment data sets used to build it, thus providing a fair test of its ability to make useful predictions across these species and experimental conditions.

The model's predictive validity was further tested through new experiments in male B6C3F1 mice, conducted at the University of Colorado Medical School in Denver by Drs. R. Irons and W. Stillman. Table 3 summarizes the experimental design used. Five mice were sacrificed at each "x" and 50 mg/kg of CP was administered by i.p. injection into surviving mice at each "D". Rows 1-3 represent three treatment groups, receiving one, two, and three doses of CP, respectively, spaced 48 h apart. Row 0 is the control group. This design was constructed based on simulation model results, which predicted large, testable differences in CFU-GM population sizes on different days for these three dose regimens.

Table 3. Design of model validation experiment in mice.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0		X		x			x	x	x	x	x			x
1	D	X	x	x					x					
2	D		D, x	x			x	X		x	x			x
3	D		D				D, x	X	x	x	x			x

D = Administration of dose (50 mg/kg via i.p. injection).
X = Sacrifice of animals.

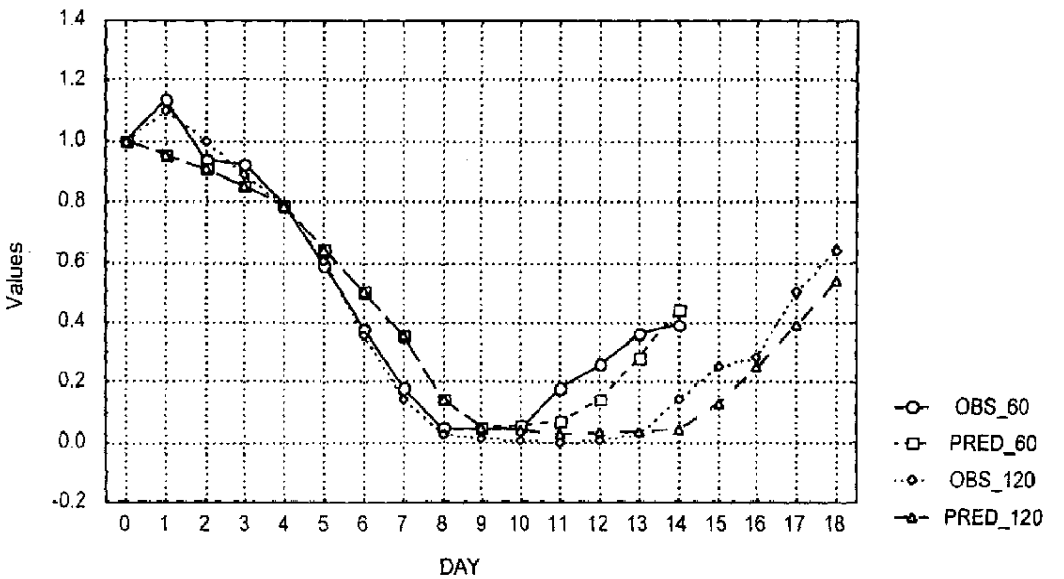

Fig. 1. Model predictions vs. clinical observations for two administered doses (60 and 120 mg/kg of CP). Experimental data from clinical trial of Buckner et al. 1972. OBS=observed values; PRED=model-predicted values.

A pilot experiment consisting of a single injection on day 1 and a 1-week follow-up with sacrifices on days 2, 4, and 7 confirmed that mice could tolerate 50 mg/kg and that CFU-GM and WBC counts looked as predicted on these days. Mice were sacrificed by cervical dislocation and bone marrow was extracted, plated, and assayed for CFU-GM stem cells using a colony-forming assay.

RESULTS

Following the validation tests, the biologically-motivated hematotoxicity model was applied to several dose scenarios to determine whether it could help to explain the phenomena in Tables 1 and 2. Scenarios examined included:

1) Stop-exposure experiments, similar to those for isoprene and benzene in Tables 1 and 2, that allocate the same total administered dose according to different time patterns to determine which create the largest predicted hematotoxic effects on CFU-GM populations.

2) Continuous infusion experiments, intended to create internal dose profiles similar to those that might arise from sustained low-level exposures to an air-borne leukemogen.

The model-based predictions for these dosing scenarios were used to draw inferences about dose-response relations that may help to explain some of the qualitative patterns noted in Tables 1 and 2 for other chemicals and end-points.

Results of model validations for humans

Buckner et al. (1972) reported clinical data for 9 human cancer patients administered 60 mg/kg of CP each via infusion, and for 7 patients each given 120 mg/kg CP as 2 60 mg/kg infusions spaced 1 day

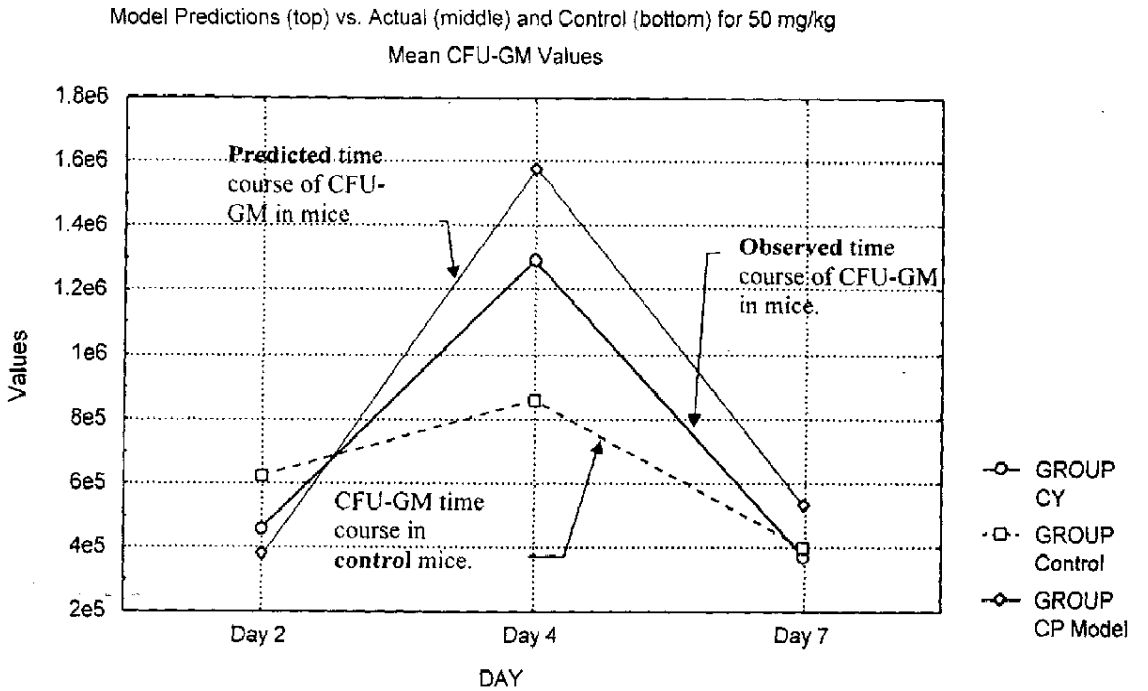

Fig. 2. Predicted vs. actual CFU-GM values in mice after one CP injection.

apart. The CP model was used to predict the time courses of WBCs for these groups of patients. Figure 1 shows the model predictions and the observed data for both groups. The results from the simulation model provide a useful approximation to the empirically observed results. In both the simulation and the clinical data, the WBC time courses are not significantly different between the 60 mg/kg and the 120 mg/kg groups for the first week following exposure. They begin to separate in the second week, with severe leukopenia persisting for over 4 d in the 120 mg/kg group before noticeable recovery begins, compared to an earlier recovery in the 60 mg/kg group. This match between predictions and observations is reassuring, especially considering that the model was developed using canine data. Model predictions match observed data similarly well throughout the duration of observations for a clinical trial in which CP was administered on four consecutive days (Nissen-Meyer and Host 1960).

Results of validation experiments in mice

Figures 2 and 3 present the main results of the validation experiments in mice. Figure 2 shows predicted vs. actual results for CFU-GM time courses in a single-injection pilot experiment. Peripheral granulocyte-macrophage counts were also predicted, and provide even better matches to observed values than CFU-GM. Since the bone marrow CFU-GM responses are both more volatile and hence harder to predict, they are the focus of this summary. Figure 3 shows the outcome of the multiple-injection experiment described in Table 3. The three panels, a, b, and c, show the observed (squares) and predicted (circles) average values of CFU-GM for mice sacrificed on different days in each of the three treatment groups. Straight-line extrapolations and interpolations are sketched among the data points. In the middle panel, for dose group 2, these segments are not connected for days 5 and 6 because their locations are uncertain. Mean values of CFU-GM counts among all animal sacrificed in each treatment group on each sacrifice day are shown. All plotted values are expressed relative to the control group values, i.e., 1 is the no-effect level.

There is enough experimental variability between replicates so that an exact match between predicted and observed values would not be expected in any single experiment. For example, the left-most panel of Fig. 3 repeats the single-injection dose regimen in Fig. 2, yet produces a different time course of observed relative CFU-GM counts in bone marrow. However, the predicted time courses are similar to the observed ones in

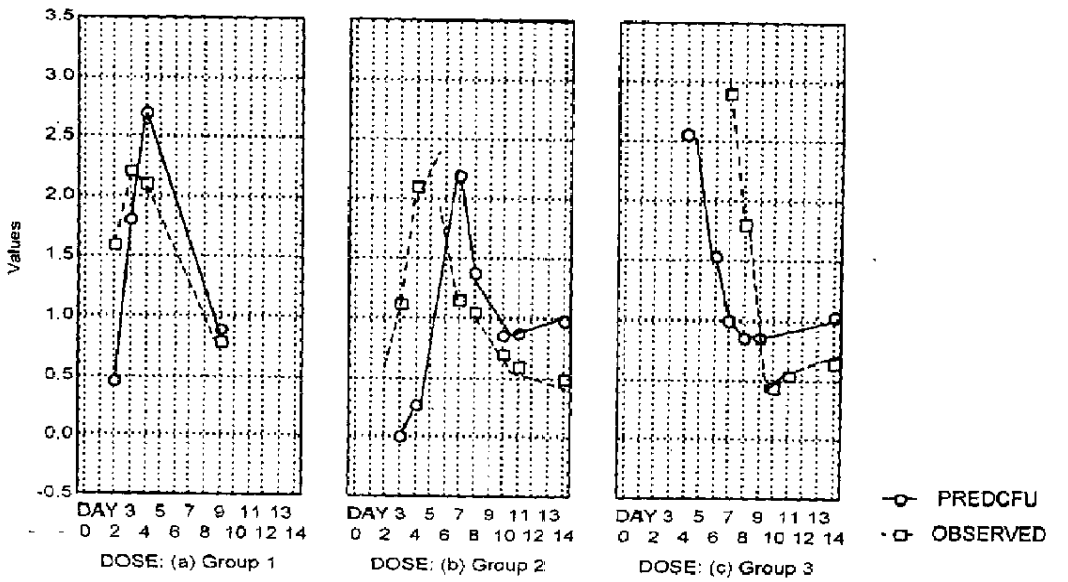

Fig. 3. Predicted vs. observed CFU-GM values in mice after (a) 1, (b) 2, and (c) 3 CP injections.

approximate magnitudes and timings of changes in CFU-GM counts. The largest discrepancies (e.g., at the left ends of the curves in Fig. 3) occur when the CFU-GM compartment is predicted to be most volatile, with population sizes changing dramatically from hour to hour. Indeed, the experiment design in Table 3 was chosen to produce just such large, fast variations so that they could easily be detected. There is rough agreement between predicted and observed values in terms of magnitude, direction of change, and approximate timing of peaks and of recovery (starting to return to pre-exposure levels), despite the experimental variability.

The model validation results in Figs. 1 and 2 suggest that the simulation model of hematotoxicity may provide useful approximate predictions in novel situations, especially for humans but less so for mice. This suggestion is strengthened by independent investigations of other researchers (Fliedner et al. 1996; Tibken and Hofer 1995), who also used an outgrowth of the Steinbach et al. (1980) model to predict CFU-GM responses in dogs and humans exposed to radiation. Their results and the new model validation experiments reported above provide empirical support for applying the simulation model to predict CFU-GM and peripheral granulocyte-macrophage responses to situations for which data are not yet available.

Results of model applications

Figure 4 examines the predicted effects of repeated infusions of CP, selected to roughly mimic the experimentally-observed GM-lineage effects of two different concentrations of benzene inhaled for 6 h/d for different amounts of time (Green et al. 1981b). The exposure corresponding to 10 ppm (μL/L) for 10 weeks (or more) has only a relatively small predicted effect on mouse bone marrow CFU-GM (curve 2). Yet, an algebraically equal (or smaller) exposure of 100 ppm (μL/L) for 6 h/d for 1 week has a much larger predicted effect on CFU-GM (curve 1), as measured by depth of nadir and height of zenith. Such predictions agree with and may help to explain experimental observations (e.g., Green et al. 1981b).

Other simulation experiments produced the following conclusions:

1) A dose of CP administered over 8 h creates a larger hematotoxic effect than the same dose administered over 120 h or 480 h, as measured by depth of nadir or maximum height of the recovery curve of CFU-GM. The 8-h administration leads to a profound initial depression in CFU-GM cells, followed by a rapid rebound and overshoot as a result of compensating proliferation. Such a pattern might create a higher risk of leukemia than the other dosing scenarios if the proliferation amplifies a population of stem cells that has

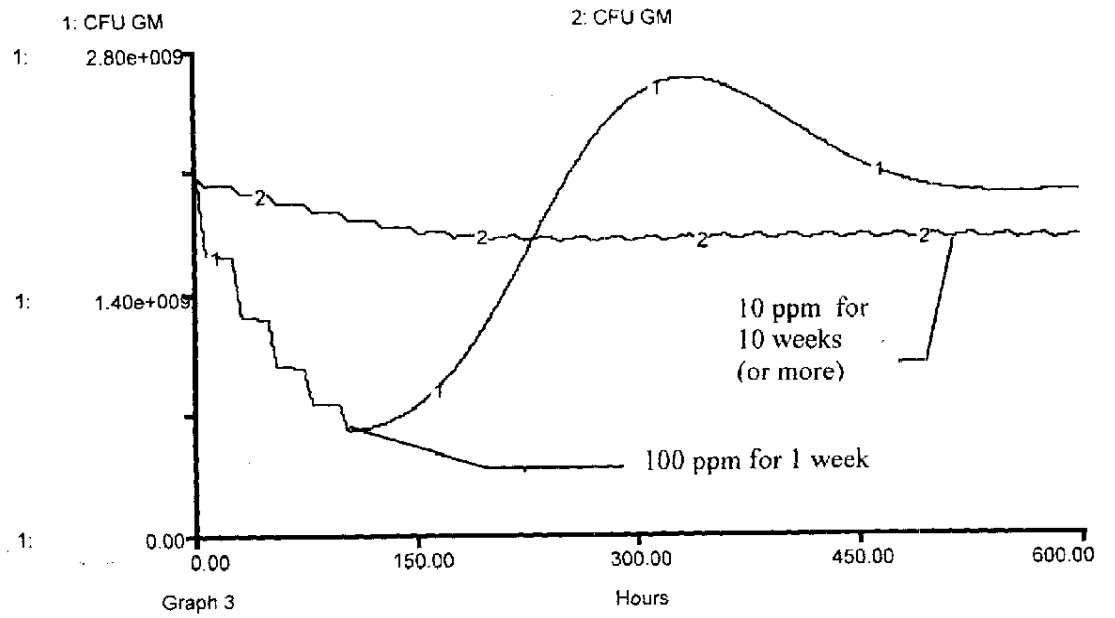

Fig. 4. Equal administered doses may create different hematotoxic responses (ppm = μL/L).

just been enriched with early HPCs (Irons and Stillman 1996).

2) Doubling the duration of exposure from 240 h to 480 h less than doubles the simulated hematotoxic response. In effect, the hematopoietic system is able to partially recover during exposure (provided the dose rate is small enough to be tolerated without driving CFU-GM toward zero). During sustained dosing at low concentration levels, the number of early CFU-GM cells approaches a stressed-equilibrium level that is somewhat higher than normal. The nadir during the initial transient response is not very low (i.e., the normal stem cell population is not much depleted) and the recovery period is comparatively lengthy. Thus, the post-dosing proliferation may be expected to occur in a population that has not been as greatly enriched with at-risk stem cells as in the 8-h dosing scenario. Indeed, if repair or elimination of inappropriately recruited stem cells and their progeny takes place at a fast enough rate, then the 240-h exposure (leading to a zenith of proliferation about 2 weeks after dosing begins) might plausibly be more risky than the 480-h exposure.

3) Hematotoxic response (e.g., as indicated by height of zenith) is not a simple monotonic function of administered concentration. For example, suppose that dosing is continued until a steady-state equilibrium is reached. If the dose rate (e.g., administered concentration) is sufficiently small, then the predicted equilibrium size of the CFU-GM pool is greater than in the absence of dosing and it increases as dose rate increases. At higher concentrations, however, the CFU-GM pool size falls below its normal unstressed level and decreases further as dose rate increases.

If other organ systems and stem cell populations undergo similar feedback control processes in responding to chemical carcinogens, then similar qualitative characteristics might be expected for the dynamic dose-response relations.

Table 4 summarizes recent findings for several chemicals that suggest that, when cytotoxicity-mediated cell proliferation plays an essential role in experimentally-observed carcinogenesis, the dose-response pattern is typically non-linear at low doses and may exhibit hormesis (Calabrese et al. 1993). The model described in this paper offers a detailed explanation/prediction of this pattern in terms of the predicted dynamics of bone marrow stem cell (CFU-GM) responses to myelotoxic agents such as CP.

DISCUSSION

The time patterns of hematopoietic responses generated by the CP model predict several phenomena that are inconsistent with simpler dose-response models but that occur in practice. These include:

Table 4. Recent evidence on cytotoxicity and dose-response relations for several chemicals.

Chemical	Main result	Conjectured mechanism	Comments	Reference
Benzene	"Excess risk due to benzene exposure may be nonexistent or negative at sufficiently low doses." PBPK and hematotoxic models both support this nonlinear pattern.	Benzene exposure may increase s-MDS/leukemia risk by cytotoxic action on HPC and normal stem cell populations followed by compensating proliferation.	A simulation model predicts that at low concentrations, benzene increases stem cell counts in the bone marrow, instead of reducing them.	Cox 1996 See also Farris et al. 1997 and Cronkite et al. 1989
Caffeic acid	Forestomach and kidney hyperplasia in rats and mice are nonlinear ("J-shaped or U-shaped") functions of dietary concentrations.	Hyperplasia is considered the probable cause of cancer in the two target organs (forestomach and kidney).		Lutz et al. 1997
Chloroform	"The dose-response relationship for chloroform-induced tumors in rats and mice is nonlinear" (J- or U-shaped).	Cytotoxicity leading to regenerative cell proliferation is "the likely mode of action for the carcinogenic effects of chloroform".	Chloroform lacks clear direct *in vivo* or *in vitro* genotoxicity.	Golden et al. 1997. See also Butterworth et al. 1994
Diesel exhaust	The dose-response relation for DE-induced lung tumors is highly nonlinear (with an apparent threshold or steep J or U shape).	Lung tumors in rats occur only if exposures "over-burden" the lung, causing repetitive irritation/ injury and compensating cell proliferation.		Valberg and Watson 1996
Formaldehyde	"Formaldehyde induces nonlinear, concentration-dependent... cell proliferation and DNA-protein cross-link formation." (Monticello and Morgan 1994)	"The nonlinearity observed in formaldehyde-induced rodent nasal cancer is consistent with a high-concentration effect of regenerative cell roliferation..."	"The concentration-dependent increases in cell proliferation correlated strongly with the tumor response curve".	Monticello and Morgan 1994. See also Monticello et al. 1996 and Lutz 1991.
TCE	"The mechanism of carcinogenesis of TCE... is nonlinear: very high doses, sufficient to cause cellular necrosis, are necessary."	"Malignancy arises from repeated cycles of necrosis and regeneration with the ultimate emergence of hyperplasia and then neoplasia."		Steinberg and DeSesso 1993. See also Clewell et al. 1995.

1) Responses that increase less than proportionally when exposure durations are increased while holding administered concentration constant.
2) Nonmonotonic relations between concentration and response, suggesting that the relation between concentration and response may be different at very low concentrations than it is at higher concentrations.
3) Response patterns that depend not only on the total dose (AUC of administered dose), but also on how it is administered over time. Specifically, reducing exposure duration while proportionally adjusting exposure concentration can lead to larger responses (deeper nadir, higher and quicker zenith) for the same total administered doses.

These simulated phenomena are consistent with some of the complexities observed in real dose-response patterns from stop-exposure experiments, as mentioned in the Introduction.

These results suggest possible explanations for the apparent anomalies noted in Tables 1 and 2, including the fact that larger cumulative doses may create smaller effects. The explanation relies on the nonlinearity of stem cell dynamics in response to cytotoxic stresses. Although the CP model is specific to hematopoietic cell populations and leukemogenesis, it is tempting to speculate that similar nonlinear dynamics and feedback control loops may help to explain the observed anomalies in other experimental systems, such as those in Table 4.

In addition to predicting specific response patterns, the CP model provides potentially useful qualitative insights into the dynamics of hematopoietic responses. For example, simulation experiments in which two doses are separated by different amounts of time show that the hematopoietic system response smooths rapid transients in exposure inputs. In other words, it

responds slowly enough (on a time scale of days to weeks) so that doses spaced closely (hours to a day or two apart) create essentially the same impact as a single combined dose. Thus, for example, if it is desired to keep both the depth of the initial nadir of WBCs and the height of the subsequent peak small, then increasing the duration of the recovery period between the two successive doses from 1 d to 4 d would make relatively little difference. Allowing a week or 10 d instead of 4 d between successive doses would make a relatively large difference. Similarly, reducing daily occupational exposure and the concentration each by one half are expected to have a larger impact than reducing weekly and yearly exposure time by one half.

CONCLUSIONS

The type of dynamic dose-response model developed here for CP may help to design exposure regulations and guidelines to protect human health more effectively than standards based on AUC or concentration. Guidelines that take into account the importance of exposure timing might be less burdensome than some current standards that assume (incorrectly, if the simulation experiments reported here are an accurate guide) that long-duration, low-concentration exposure scenarios are equivalent in risk to brief, high-concentration exposures. The dynamic simulation approach described in this paper can help to quantify the extent to which realistic dynamic dose-response patterns are likely to differ from those predicted by simpler dose-metric models.

Acknowledgments—The author thanks Drs. Mary Paxton, Michael Bird, Patrick Beatty, and Robert Schnatter. This work was supported in part by the American Petroleum Institute (API).

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